Flagellin induces innate immunity in nonhost interactions that is suppressed by *Pseudomonas syringae* effectors

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*Arabidopsis NONHOST1* (*NHO1*) is required for limiting the in planta growth of nonhost *Pseudomonas* bacteria but completely ineffective against the virulent bacterium *Pseudomonas syringae* pv. *tomato* DC3000. However, the molecular basis underlying this observation remains unknown. Here we show that *NHO1* is transcriptionally activated by flagellin. The nonhost bacterium *P. syringae* pv. *tabaci* lacking flagellin is unable to induce *NHO1*, multiplies much better than does the wild-type bacterium, and causes disease symptoms on *Arabidopsis*. DC3000 also possesses flagellin that is potent in *NHO1* induction, but this induction is rapidly suppressed by DC3000 in a type III secretion system-dependent manner. Direct expression of DC3000 effectors in protoplasts indicated that at least nine effectors, HopS1, HopA1I, HopA2I, HopT1-1, HopT2-2, HopAA1-1, HopF2, HopC1, and AvrPto, are capable of suppressing the flagellin-induced *NHO1* expression. One of the effectors, HopA1I, is conserved in both animal and plant bacteria. When expressed in transgenic *Arabidopsis* plants, HopA1I promotes growth of the nonpathogenic hrpL- mutant bacteria. In addition, the purified phytotoxin coronatine, a known virulence factor of *P. syringae*, suppresses the flagellin-induced *NHO1* transcription. These results demonstrate that flagellin-induced defenses play an important role in nonhost resistance. A remarkable number of DC3000 virulence factors act in the plant cell by suppressing the species level defenses, and that contributes to the specialization of DC3000 on *Arabidopsis*.

Nonhost resistance refers to resistance shown by an entire plant species to a specific parasite (1). This resistance is expressed by every plant toward the majority of potential phytopathogens and differs from the cultivar-level resistance conditioned by gene-for-gene interactions (2, 3). Plant defenses can be induced by “general elicitors” of pathogen or plant origin, including oligosaccharides, lipids, polypeptides, and glycoproteins (4). However, a role of these elicitors in plant disease resistance in a natural setting is often difficult to establish, because plants’ responses to elicitors do not differentiate resistant and susceptible plants. Many of the elicitors are now known as pathogen-associated molecular patterns (PAMPs). The best-characterized PAMP known to activate innate immunity in plants is flagellin from *Pseudomonas* bacteria (5). A conserved N-terminal peptide of flagellin, flg22, is a potent elicitor of defense responses in tomato and *Arabidopsis* (5, 6). In *Arabidopsis*, flg22 is perceived by FLS2, a receptor-like kinase that activates downstream events through a MAP kinase cascade (7, 8). Pretreatment of *Arabidopsis* with flg22 peptide not only globally induces defense gene expression, but also protects plants from subsequent infection of the virulent DC3000 (9). *Arabidopsis* plants lacking FLS2 exhibit enhanced disease susceptibility to DC3000 under certain circumstances (9). Although these studies elegantly demonstrated the functional significance of flagellin-sensing in plant defense, whether flagellin-signaling plays a role in the species level resistance remains unknown.

The bacteria enter plants through natural openings such as stomata or wounds and proliferate in the intercellular spaces. A major bacterial pathogenesis mechanism is mediated by the so-called type-III secretion system (TTSS), through which Gram-negative bacteria inject a repertoire of effectors into host cells (10). Type III effectors play an important role in bacterial pathogenesis. In *Pseudomonas syringae*, a growing number of effector gene products, such as *avrRpt2*, *avrRpm1*, *virPphA*(*hopAB1*), *avrPto*, and *hopAB2*(*avrPtoB*), are known to contribute to virulence (11–16). *avrRpt2*, for example, suppresses plant PR gene expression and interferes with the RPM1-specified resistance (11). *avrPtoB*, *hopXI*(*avrPphEmo*), *hopAM1*(*avrPpiBpao*), *hopAO1*(*hopPtoD2*), *hopE1*(*hopPtoE*), *hopF2*(*hopPtoF*), *hopF1*(*avrPphF*), and *hopN1*(*hopPtoN*) all appear to suppress cell death in plants (17, 10). In addition to type III effectors, certain *P. syringae* strains, including DC3000, produce the phytotoxin coronatine, which also plays a role in bacterial virulence (18). A role of TTSS or coronatine in overcoming nonhost resistance has not been examined closely.

In previous studies, we showed that the *Arabidopsis* *NHO1* gene is required for resistance to multiple strains of nonhost *P. syringae*, but completely ineffective against DC3000 (19). Interestingly, *NHO1* transcripts are induced by the nonhost strains, but suppressed by DC3000 (20). This suppression is apparently of functional significance, because plants overexpressing *NHO1* exhibit enhanced resistance to DC3000 (20).

Here, we show that the flg22 peptide strongly induces the transcription of *NHO1*. A *P. syringae* pv. *tabaci* (Pub) strain, to which *Arabidopsis* is a nonhost plant, induces *NHO1* in a flagellin-dependent manner. A Pub strain lacking the flagellin gene *flgC* elicits disease symptoms and multiplies in *Arabidopsis* plants, demonstrating that flagellin signaling contributes to nonhost resistance. In contrast to nonhost bacteria that give a prolonged induction of *NHO1*, DC3000 only transiently induces *NHO1* transcription, also in a flagellin-dependent manner. Although the wild-type DC3000 rapidly suppresses the *NHO1* induction, DC3000 mutant strains defective in TTSS are diminished in their ability to suppress *NHO1*. Expression of the DC3000 effectors HopS1, HopAI, HopAFI, HopT1-1, HopT2-2, HopAA1-1, HopF2, HopC, and AvrPto in the plant cell blocks the *NHO1* induction by flg22. In addition, purified coronatine suppresses the flg22- and *P. syringae* pv. *phaseolicola* (*Pph*)-induced *NHO1* expression. Furthermore, expression of HopAI in transgenic plants promotes nonpathogenic bacterial growth. Together, these results demonstrate the importance of flagellin-induced innate immunity mechanism in nonhost resis-
tance and a role of DC3000 virulence factors in suppressing the flagellin-induced innate immunity.

Materials and Methods

Construction of NHO1–LUC Reporter Line. A 1.8-kb NHO1 promoter sequence was PCR-amplified from Col− genomic DNA with the following primers: 5′-CAGTCGACCTTCTTACAGTCTCAGCACA
CATG-3′ and 5′-TCCCGGGGTTAAGGTTAAGAAAC
GATGCT-3′. The PCR product was digested with Sall and Smal and cloned into a modified pH1121 vector with the LUC reporter gene (21). The NHO1–LUC construct was introduced into Agrobacterium tumefaciens strain GV3101 and transformed into Col-0 plants by floral-dipping (22). The T4 progeny of a selected homozygous transgenic line with a single insertion was used for all experiments.

Bacterial Strains and Bacterial Growth Assay. Bacterial strains used in this study include DC3000, Pph NPS3121 (19), Pab 6505 wild-type and 6505 flf C mutant (23), DC3000 hrr A mutant (24), DC3000 hrr A and hrc C mutants (25, 26), and DC3000 flf C mutant (previously referred to as fla A; ref. 27). Bacteria were grown overnight at room temperature in King’s medium B with appropriate antibiotics, precipitated, washed twice with double distilled H2O (ddH2O) and diluted to the desired concentration with ddH2O for plant inoculation. Bacteria used for growth assay was diluted to 106 colony-forming units (cfu)/ml and syringe-infiltrated into young and fully expanded Arabidopsis leaves. All experiments were repeated at least twice with similar results.

Luciferase Activity Assay. Bacterial cultures used for luciferase activity assay were diluted with 0.2 mM luciferin to 106 cfu/ml and syringe-infiltrated into Arabidopsis leaves. The leaves were removed from plants at the indicated time points and sprayed with 1 mM luciferin containing 0.01% Triton X-100. Luminescence images were captured by using a low-light image system, and relative luciferase activity was calculated with WINVIEW software (Roper Scientific, Trenton, NJ) (21).

Flagellin and Coronatine Treatment. Polypeptides containing 22 conserved N-terminal residues of flagellin from P. aeruginosa, Pab, and Agrobacterium tumefaciens were synthesized by Bio-Synthesis (Lewisville, TX) as the following: flg22P.aeruginosa, QRLSTGSRIN
SARKDDAAGLOIA; flg22A. tumefaciens, ARVSSGLRVGDA
DAAAYWSIA; and flg22P. tabaci, TRLSSGLKINSKADDAAG
LOIA. Flg22 peptides were dissolved in ddH2O and diluted to 1 µM with 0.2 mM luciferin before inoculation. Coronatine (kindly provided by Carol Bender, Oklahoma State University, Stillwater) was dissolved in ddH2O and diluted to 100 ng/ml with 0.2 mM luciferin before inoculation.

Construction of Effector Gene Expression Plasmids. A transient expression vector pUC19-35S-FLAG-RBS containing the cauliflower mosaic virus 3S promoter, 3× FLAG, and a Rubisco Small Subunit terminator (Y. Zou and J.M.Z. unpublished results; GenBank accession no. DQ077692) was used for transient expression of effector genes in protoplasts. The effector genes were PCR-amplified with primers listed in Table 1, which is published as supporting information on the PNAS web site. After restriction digestion, the avrPto PCR product was inserted between the XhoI and SpeI sites of pUC19-35S-FLAG-RBS, resulting in the 35S-AvrPto construct. Other effector genes were inserted between XhoI and Csp45I of pUC19-35S-FLAG-RBS, resulting in 35S-Effector-FLAG constructs.

Protoplast Transfection Assay. Protoplasts were isolated from 6-week-old NHO1–LUC plants according to Sheen (http://genetics.mgh.harvard.edu/sheenweb). Protoplasts were transfected with either an effector construct or the empty vector, and incubated in 0.4 M mannitol and 1 µM flg22P. tabaci for 12 h. LUC activity was measured after adding 50 µM luciferin to the transfected protoplasts.

Construction of Estradiol-Inducible hopA11 Expression Plants. The HopA11-FLAG fragment was excised from the 35S-HopA11-FLAG plasmid with XhoI and SpeI and inserted into pER8 (28). The construct was transformed into Arabidopsis plants (Col-0) by Agrobacterium-mediated transformation. Transgenic plants were selected on Murashige and Skoog plates containing hygromycin. For hopA11 induction, plants were sprayed with 25 µM estradiol containing 0.02% silwet L-77.

Supporting Information. For further details, see Supporting Text, Table 1, and Figs. 7–9, which are published as supporting information on the PNAS web site.

Results

Flagellin Induces NHO1 Transcription. To further investigate the regulation of NHO1 expression in response to Pseudomonas bacteria, an NHO1–LUC reporter line was constructed. Consistent with the expression of endogenous NHO1 mRNA (20), the NHO1–LUC expression in a homozygous reporter line was strongly induced by Pph, but not DC3000 (Fig. 1a). Detailed analysis revealed a transient NHO1–LUC induction 3 h after DC3000-inoculation, but the NHO1–LUC expression returned to the baseline by 12 h (Fig. 1b). In contrast, Pph induced a strong and sustained expression of NHO1–LUC. The strong induction by nonhost bacteria is not strain-specific, because another nonhost strain Pab also induced NHO1–LUC to a high level (Fig. 2a).

We previously hypothesized that a PAMP derived from the nonhost Pseudomonas bacteria induces the expression of NHO1 (20). Flagellin is a well known PAMP that induces innate immune responses in plants and animals. Therefore, we tested whether flagellin induces the NHO1–LUC reporter gene. Flg22 peptides corresponding to P. aeruginosa, A. tumefaciens, and P. syringae pv. tabaci were tested for their ability to induce NHO1–LUC. Fig. 2 shows that the active peptide flg22P. aeruginosa was fully capable of inducing NHO1–LUC. Flg22P. tabaci was similarly active in NHO1–LUC induction (see Fig. 6c). In contrast, flg22A. tumefaciens, which is inactive in plant immune response induction (5), was unable to induce NHO1–LUC (Fig. 1c).

Flagellin Is Required for NHO1 Induction and Resistance in Nonhost Interaction. If flagellin is required for the NHO1 induction by a nonhost bacterium, then bacteria lacking flagellin should be defective in NHO1 induction. A Pab mutant strain lacking the flagellin gene flc− (23) poorly induced the NHO1–LUC expression (Fig. 2a), indicating that flagellin is largely responsible for the observed induction of NHO1 by this bacterium. To test whether flagellin contributes to nonhost resistance in Arabidopsis plants, the flc− mutant was compared with the wild-type Pab for disease symptoms and bacterial growth in planta. Fig. 2b shows that flc− caused visible disease symptoms on Arabidopsis. In contrast, the wild-type bacterium caused no visible symptoms. The mutant bacteria multiplied at least 10-fold 4 days after inoculation, whereas the wild-type Pab failed to multiply in the 4-day period (Fig. 2c). Together, these data demonstrate that flagellin is a major PAMP responsible for the induction of NHO1 and resistance to this nonhost P. syringae bacterium.

Transient Induction of NHO1 by DC3000 Requires Flagellin. The possibility that transient NHO1–LUC induction by the wild-type DC3000 depends on flagellin was also tested. The flc− mutant of DC3000 failed to induce NHO1–LUC at any tested time point after inoculation (Fig. 3a). In addition, wild-type DC3000 bacteria killed by exposure to kanamycin before inoculation also induced a strong and sustained NHO1–LUC expression, whereas the flc− bacteria
killed by kanamycin did not induce NHO1–LUC (data not shown). Bacterial growth assay indicated that the wild-type and fliC/H11002 mutant of DC3000 grew similarly when infiltrated into Arabidopsis plants (Fig. 3b). The two strains also caused indistinguishable disease symptoms (data not shown). These results demonstrate that, like Ptab, DC3000 flagellin is fully capable of inducing NHO1. However, unlike Ptab, the response to DC3000 flagellin is abrogated and does not result in resistance in the plant.

**TTSS Is Essential for DC3000 to Suppress NHO1.** The lack of sustained NHO1–LUC induction by DC3000 flagellin is consistent with our hypothesis that this bacterium actively suppresses the NHO1-mediated nonhost resistance (19). Therefore, a role of DC3000 virulence/pathogenicity genes in the active suppression of NHO1 was tested. Fig. 4a shows that DC3000 strains lacking the TTSS structure genes hraP4 and hrcC induced much greater NHO1–LUC expression compared with the wild-type DC3000, indicating that TTSS is largely responsible for the suppression. The DC3000 mutant lacking the regulatory gene hrl encodes an even stronger induction than did hrl– and hrc– mutants (Fig. 4a). The strength and kinetics of the hrl– mutant-induced NHO1–LUC expression resemble those of Ptab (Figs. 4b and 1b). hrl encodes a sigma factor that regulates both TTSS and coronatine biosynthetic genes through the hrl box (29). These results demonstrate that TTSS is essential for DC3000 to suppress the NHO1 expression.

**Type III Effectors Suppress NHO1 Expression.** The hypothesis that type III effectors suppress NHO1 expression was systematically tested by using a protoplast-based transient assay. Protoplasts were isolated from plants carrying the NHO1–LUC reporter and transfected with constructs carrying DC3000 effector genes under the control of the cauliflower mosaic virus 35S promoter. A total of 19 effectors were tested (Table 1; www.pseudomonas-syringae.org). Most of these
effectors were selected because their function in virulence had not been reported previously. For control, protoplasts were transfected with an empty vector. The transfected protoplasts were subsequently induced with

\[ \text{flg}22 \] Ps.tabaci

induced NH01–LUC in protoplasts transfected with empty vector compared to uninduced protoplasts, recapitulating the NH01–LUC induction observed in intact leaves. Transfection of nine effector genes, hopS1, hopAI1, hopAF1, hopT1-1, hopT1-2, hopAA1-1, hopF2, hopC1, and avrPto, strongly reduced the flagellin-induced NH01 expression in repeated experiments. Among these, hopAI1, hopT1-1, hopAA1-1, hopF2, and hopC1 completely abolished the NH01 induction. Other effector genes did not show a consistent effect on NH01 induction. These results indicate that almost 50% of the tested DC3000 effectors are functionally redundant and suppress the flagellin-induced NH01 expression.

Southern blot analysis was carried out to determine whether any of these nine effector sequences exist in the two nonhost strains used (Fig. 9). Not all of the nine effectors described in this work are unique to DC3000. HopAA1 is encoded by the conserved effector locus that exists in all known P. syringae pathovars (30). Southern blot analysis indicated that the hopT1-1 and hopAA1 sequences exist in Ptab, whereas the hopAF1, hopT1-2, and hopAA1 sequences are present in Pph. Thus, it appears that the delivery of a few of these effectors to the bacterium is not sufficient for the suppression.

**HopAI1 Promotes Parasitism in Plants.** To determine whether any of the tested effectors promote virulence, a FLAG-tagged hopAI1 was introduced into Arabidopsis plants as a stable transgene by using an estrodial-inducible system (28). This effector was chosen because it shares 35% identity with the Salmonella enterica serovar typhi

\[ \text{murium} \]

VirA, a mouse killing factor (ref. 31 and Fig. 1). A search of the GenBank database indicated that similar proteins also exist in Salmonella choleraesuis, Shigella flexneri, and Chromobacterium violaceum. Fig. 5a shows that induced expression of hopAI1 in a transgenic line exhibited chlorosis, reminiscent of disease symptoms. The expression of hopAI1 also enhanced the growth of the hrlP

\[ \text{mutant} \]

bacteria by at least 30-fold (Fig. 5b). Similar results were observed in six primary transgenic plants (Fig. 8). These results indicate that the suppression of NH01 by HopAI1 is relevant to the virulence function.

The role of hopAI1 in NH01-suppression was further tested by using a DC3000 mutant strain carrying truncated hopAI1. Consis-
Coronatine Partially Suppresses NH01 Expression. Previous work suggested that both TTSS and the phytotoxin coronatine modulate the expression of a similar set of plant genes (21, 30). This suggestion prompted us to test whether coronatine also contributes to the observed suppression of NH01. Fig. 6 a and b show that coinfiltration of purified coronatine diminished the NH01–LUC expression induced by flg22\textsubscript{P.s.tabaci} or Pph. However, a DC3000 mutant that is blocked in the synthesis of coronatine was only marginally compromised in NH01–LUC suppression (data not shown). Together, these results suggest that coronatine plays a minor role in NH01 suppression. A role of coronatine and the requirement of COI1 in NH01 suppression (20) indicate that jasmonate signaling may play a role in NH01 suppression. Consistent with this possibility, exogenous application of methyl jasmonate partially suppressed the Pph-induced NH01–LUC expression (data not shown).

Discussion

The molecular basis of nonhost resistance is poorly understood. It is speculated that PAMP-induced innate immunity plays an important role in the species level resistance, but direct evidence is lacking (4). The results presented here show that flg22, a known PAMP, mimics nonhost bacteria and induces the expression of NH01. In contrast, the inactive peptide flg22\textsubscript{A.tumefaciens} is unable to induce NH01. Thus, the induced expression of the nonhost resistance gene NH01 is a typical PAMP-mediated innate immune response.

Recent results showed that Pseudomonas bacteria carry at least two additional PAMPs, a cold-shock protein and elongation factor-TU, both inducing defense responses in plants (32, 33). The results presented here indicate that flagellin is the primary PAMP in P\textit{tab} responsible for NH01 induction, because the flg\textsuperscript{C} mutant strain is largely inactive in NH01 induction. The induction of NH01 is likely of functionally importance, because Arabidopsis plants overexpressing NH01 display enhanced resistance to DC3000 (20). The P\textit{tab} strain lacking flg\textsuperscript{C} gains partial virulence on wild-type Arabidopsis when directly infiltrated into leaves. This strain also displays enhanced virulence on tomato plants (23). It should be noted that the flg\textsuperscript{C} mutant is not fully pathogenic on Arabidopsis. One plausible explanation is that PAMPs other than flagellin also contribute to species level resistance (9). Nevertheless, these results demonstrate that flagellin plays a critical role in eliciting nonhost resistance.

Although nonhost resistance is effective to the vast majority of potential pathogens, it is breached by a small number of pathogens, presumably because the latter has evolved specialized virulence mechanisms that enable them to successfully overcome this resistance. Flagellin is highly conserved among Pseudomonads, including DC3000, which is virulent on Arabidopsis. NH01–LUC reporter assay revealed a transient induction by DC3000, and this induction is flagellin-dependent. The induction is quickly suppressed within 6 h after inoculation, and coincides with the in planta expression of type III genes in DC3000 (34). We previously hypothesized that DC3000 suppresses NH01 by using type III effectors (20). Indeed, the hrp\textsuperscript{A}, hrc\textsuperscript{C}, and hrp\textsuperscript{L} mutants of DC3000 all induce NH01–LUC to a much greater level than does the wild-type strain.
Most importantly, direct expression of nine DC3000 effector genes in the plant cell or exposure to purified coronatine strongly suppresses the flag22-induced expression of NHO1–LUC, providing direct evidence that type III effectors suppress the flagellin-induced immune responses. These observations are consistent with the knowledge that exogenous flagellin only protects Arabidopsis plants against DC3000 when applied 1 day before the bacterial inoculation. However, when infiltrated simultaneously with the DC3000 bacterium (9). Together, these results provide strong evidence that a major target for DC3000 is innate immunity that acts at the species level to limit nonhost Pseudomonas bacteria. Consistent with the role of DC3000 TTSS in overcoming species level resistance, recent work shows that the DC3000 TTSS actively suppresses and tolerates the production of antimicrobial root exudates that are inhibitory to nonhost bacteria, although which effector(s) does so remains to be determined (35). The ability of a bacterium to overcome the species level resistance may represent a major evolutionary step that enables a P. syringae bacterium to colonize on a new host species.

The results presented here indicate that a surprisingly large proportion of the DC3000 effectors possesses the ability to suppress the flagellin-induced NHO1 expression. Among the nine effectors that suppress NHO1 expression, at least HopA11 and AvrPto are capable of promoting nonpathogenic bacterial growth when expressed in plants (36). HopA11 shares significant homology with virulence proteins of animal bacteria. This finding raises an intriguing possibility that flagellin-induced innate immunity in the host is similarly targeted by diverse pathogenic bacteria. Expression of AvrPto in the plant also suppresses callose deposition induced by the hrcC mutant bacteria (36). Because callose deposition can be induced by flagellin (5), AvrPto might suppress cell wall defense and NHO1 expression through a common step required for flagellin signaling. A recent report shows that AvrRpt2 and AvrRpm1 can suppress flagellin-induced callose deposition when directly expressed in plants (37). These observations reinforce the notion that flagellin-induced defenses are targeted by diverse effectors, although they do not appear to share a conserved biochemical function.

A large number of P. syringae effectors have been shown to target various host defenses including callose deposition, defense gene expression and cell death induced by gene-for-gene interaction or nonhost interactions (17). Often, the defense suppression by an individual effector is revealed either when the latter is directly expressed at a high level in the plant cell or delivered along with other effectors in the bacterium. It remains to be determined whether these effectors, when individually delivered by P. syringae, are sufficient to suppress host defenses. It is possible that a successful defense suppression by a bacterium requires synergistic action of a large set of the bacterium-delivered effectors. For instance, conserved effector locus (CEL), which exists in all P. syringae, is required by DC3000 for pathogenicity and suppression of callose deposition in Arabidopsis (38). However, the vast majority of P. syringae is nonpathogenic on Arabidopsis. Thus, the function of CEL-encoded effectors is likely to be assisted by other effectors unique to DC3000. Similarly, several effectors activate COI1-dependent gene expression when delivered by DC3000 but not when delivered by Pph (21). DC3000 type III effectors and coronatine act synergistically to modulate the JA signaling in Arabidopsis (21, 39). These may explain why some of the nine effector sequences carried by Pab and Pph do not appear to suppress the NHO1 expression. It may be that the suppression of the flagellin-induced expression of NHO1, which is known to involve the JA signaling pathway (20), requires a synergistic activity from a large set of these effectors and coronatine that target the JA signaling pathway.

The RIM1-interacting protein RIN4 was shown recently to negatively regulate the flagellin-induced callose deposition (35). RIN4 also interacts with AvrRpt2 and AvrRpm1 (40–42). AvrRpt2 is a cysteine protease that cleaves RIN4, leading to the degradation of RIN4 (43), whereas the AvrRpm1 interaction results in the phosphorylation of RIN4 (40). It is suggested that RIN4 and/or RIN4-associated proteins are manipulated by these two effectors to suppress the flagellin-induced cell wall defense (35). It remains to be shown whether and how the AvrRpt2-mediated degradation of RIN4 leads to the suppression of callose deposition. An important area of future research will be to determine whether a common mechanism is used by various effectors to suppress flagellin-induced defenses.

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homologous sequences from other bacteria using CLUSTAL W. Homologous amino acid sequences are shaded by using BOXSHADE. St_VirA, *Salmonella typhimurium* VirA (accession A41481); Sc_s08402, *Salmonella choleraesuis* virulence-associated protein (accession S08402); Cv_VirA, *Chromobacterium violaceum* VirA (accession AAQ58983); Sf_OspF, *Shigella flexneri* OspF (accession AAP78969); HopAI1Psy, HopAI1 from *P. syringae pv syringae* (accession ZP00128143). Asterisks and dots denote invariant and conserved residues, respectively. Strong homology between the two *Pseudomonas* HopAI1 and animal bacterial proteins exists throughout the entire protein, except for the N-terminus where the presumed type III secretion signal resides.

**Supporting Figure 2.** HopAI1 expression in primary transgenic plants enhances bacterial growth in plants. Six primary transgenic plants were sprayed with 50 µM estradiol. 24 h after the spray, *hrpL* mutant DC3000 bacteria were inoculated into leaves, and bacterial population was determined 4 days later. Error bars indicate standard error. Western blot below the graph shows HopAI1-FLAG protein in individual transgenic plants one day after estradiol spray.
Supporting Figure 1. HopAI1 is a conserved effector in both animal and plant pathogenic bacteria. Amino acid sequence of DC3000 HopAI1pto (accession AA05440) was aligned with homologous sequences from other bacteria using CLUSTAL W. Homologous amino acid sequences are shaded by using BOXSHADE. St_VirA, Salmonella typhimurium VirA (accession A41481); Sc_s08402, Salmonella choleraesuis virulence-associated protein (accession S08402); Cv_VirA, Chromobacterium violaceum VirA (accession AAQ58983); Sf_OspF, Shigella flexneri OspF (accession AAP78969); HopAI1Psy, HopAI1 from P. syringae pv syringae (accession ZP00128143). Asterisks and dots denote invariant and conserved residues, respectively. Strong homology between

spray, hrpL- mutant DC3000 bacteria were inoculated into leaves, and bacterial population was determined 4 days later. Error bars indicate standard error. Western blot below the graph shows HopAI1-FLAG protein in individual transgenic plants one day after estradiol spray.
and \textit{avrPto} were digested with \textit{XhoI} and \textit{SpeI} to release the effector sequences and transferred to membrane. Triplicated membranes containing equal amounts of DNA were hybridized with radio-labeled genomic DNA isolated from the indicated bacterial strains. Standard hybridization was carried out at 65\textdegree{}C and membranes were washed to 0.5 X SSC at 65\textdegree{}C.